SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIAL AND METHODS.

Visualization and quantitation of the biofilm architecture. For the visualization of the biofilm architecture, biofilms were grown in a 24-well plate system modified from the procedure described by Ciazza and O'Toole (1). Briefly, overnight cultures were adjusted to an OD600 of 0.05 in fresh VBMM and grown in VBMM at 37°C and 220 rpm in 24-well microtiter plates at a 45° angle, ensuring that the bottom of the wells was at the air-liquid interface. The medium was exchanged every 8 hr. Following 5 days of growth, biofilms were stained using SYTO 40 (Invitrogen, CA), and subsequently viewed using the LSM510 confocal laser scanning microscope (CLSM) from Zeiss (Heidelberg, Germany). Quantitative analysis of confocal images of biofilms was performed using COMSTAT (2).

SUPPLEMENTARY TABLES

Table S1. Primers used.

Oligonucleotide*	Sequence (5′-3′)[#]
	RT-PCR/ PCR
PA3553for	GTCTACAACGAGGAAGCC
PA3553rev	GTTGCGGTTGAGGATCAC
brlR-RT-for	GCAACGACACCAGCACAC
brlR-RT-rev	GAAGCGTTCCCAGAGCTG
mreB-forward	CTGTCGATCGACCTGGG
mreB-reverse	CAGCCATCGGCTCTTCG
phoP-RT_for	GTCAGCGAATACCACCAC
phoP-RT_rev	GCTTCCAGTTCCTCGAAC
phoQ-RT-for	GAGCACCTGCCGGTGGAG
phoQ-RT-rev	CGGCAGGTTGAACTCCTCC
pmrA-RT-F	GAGAATACTGCTGGCCGAGGA
pmrA-RT-R	GTCCGATGTCGAGCACCAG
pmrB-RT-F	GGTCAACCTGCTGGTCGGCTTC
pmrB-RT-R	GGCTTCGCCGAAGTCCACCATG
	Cloning
phoP-SacI-for	GCGCGCGAGCTCATGAAACTGCTGGTAGTGG
phoP-EcoRI-rev	CGCGCGGAATTCTCACCGGCAGCGCTCG
phoQ-XbaI-for	GCGCGCTCTAGAGTGATCCGTTCCCTGC
phoQ-SacI-rev	CGCGCGGAGCTCTCAGACTGTAGCGAAAC
	EMSA binding assays
oprH-prom-F*	GCAGGCGACTGTAGAAAGCC
oprH-prim-R	GGTGTTTCTCCGTTCTGCGAGAG

*, primer was biotinylated
[#], restriction sites are underlined.

Table S2. COMSTAT[a]: quantitative analysis of biofilm structure[a] of *P. aeruginosa* PAO1 and *ΔphoP* mutant strains after 5 days of growth.

Strain	Total biomass ($\mu m^3/\mu m^2$)	Average thickness (μm)	Roughness coefficient	Maximum thickness (μm)	Substratum coverage (%)
PAO1	7.47 (±3.8)	9.77 (±5.4)	1.56 (±0.2)	65.28 (±11.7)	0.03 (±0.03)
ΔphoP	2.71 (±1.7)	3.52 (±2.5)	1.81 (±0.08)	49.81 (±14.1)	0.01 (±0.01)
ΔphoP/pMJT*phoP*	7.65 (±3.9)	9.17 (±4.7)	1.76 (±0.1)	84.69 (±7.1)	0.02 (±0.01)
ΔphoP/pMJT*brlR*	2.83 (±0.9)	3.12 (±0.8)	1.84 (±0.02)	51.22 (±8.5)	0.01 (±0.01)

[a] Values are means of data from multiple *z*-series image stacks for each strain taken at day 5. Experiments were carried out in triplicate. The number in parentheses indicates the standard deviation.

Table S3. Fold change in transcript levels of *brlR* in CF clinical isolates relative to PAO1 biofilms. qRT-PCR experiments were carried out in triplicate. Standard deviations are indicated.

Clinical isolate	Fold change in *brlR* transcript levels relative to PAO1 biofilms	Source/reference
A1	1.4±0.7	
A2	-1.1±0.2	
A7	-2.2±0.2	
C2	2.2±0.2	
D5	-1.8±0.4	
CF 1-2	3.9±0.7	(3)
CF 1-8	1.9±0.8	(3)
CF 1-13	8.2±0.9	(3)

SUPPLEMENTARY FIGURES

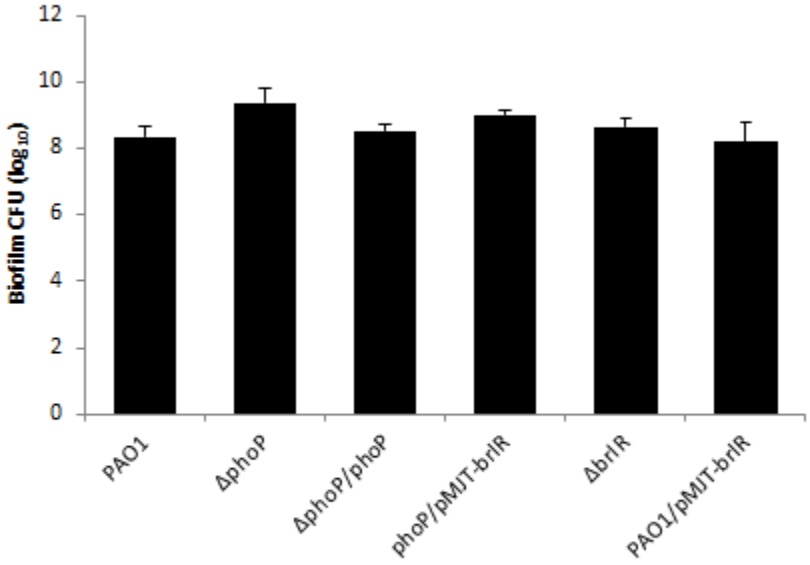

Figure S1. Biofilm CFU as determined by viability counts of *P. aeruginosa* biofilms grown for 1 day in tube reactors under flowing conditions. Biofilms were grown using VBMM. Biofilm CFU were obtained from biofilm tube reactors having an inner surface area of 25 cm^2. Experiments were repeated 6 times. Error bars denote standard deviation.

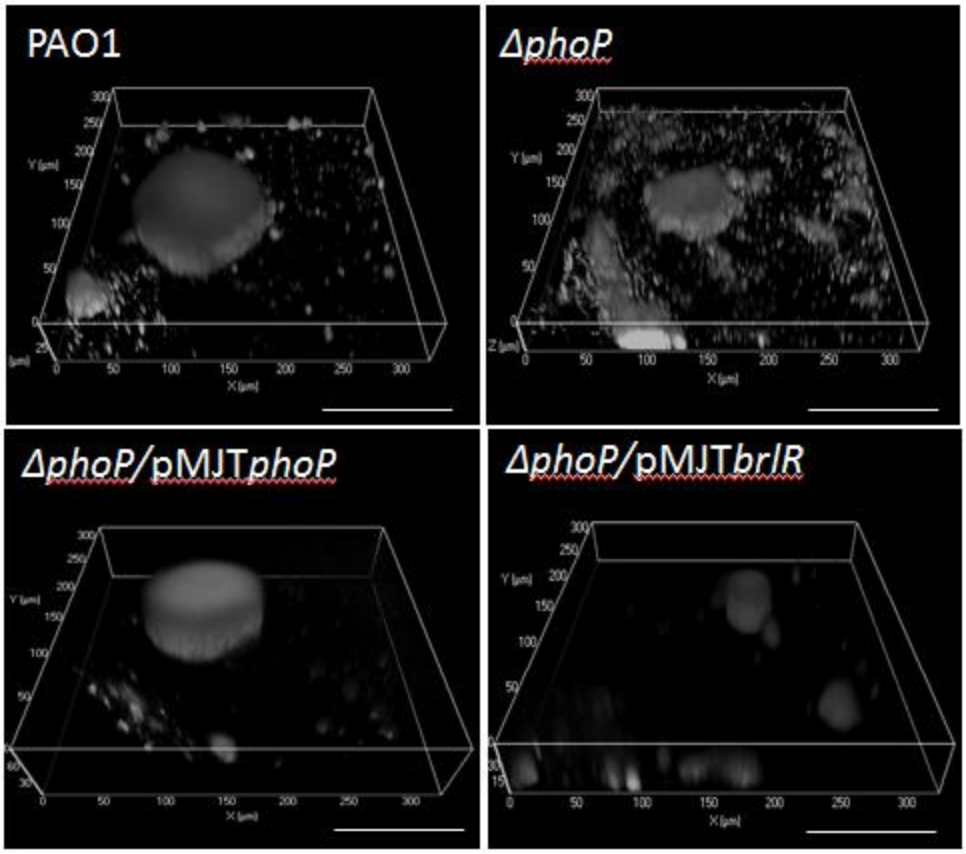

Figure S2. Confocal images of biofilms of *P. aeruginosa* wild type, *ΔphoP*, and complemented *ΔphoP* mutant strain (*ΔphoP*/pMJT*phoP, ΔphoP*/pMJT*brlR*), grown for 144 hr in 24-well plates. Biofilms were stained using SYTO40 (Invitrogen) and subsequently visualized by confocal microscopy. Experiments were carried out at least in triplicate. White bars = 100 µm.

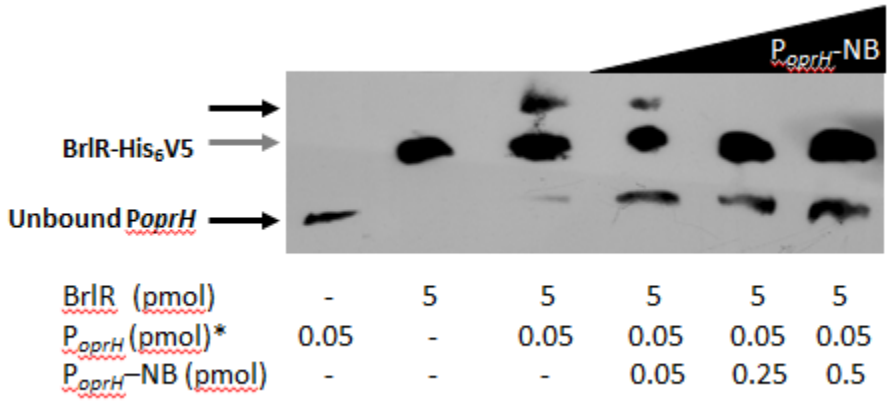

Figure S3. BrlR binds to the promoter of the *oprH-phoPQ* operon. DNA gel mobility shift assays using cell extracts obtained from *E. coli* expressing His$_6$V5-tagged BrlR (BL21/pMJT-*brlR*-His$_6$V5) *in trans.* The P*oprH* DNA fragment is 208 bp long and 0.05 pmol of the respective DNA fragment were used. The concentration of unlabeled competitor DNA (P*oprH*-NB, 0-0.5 pmol) was varied. BrlR binding to P_{oprH} was detected by immunoblot analysis using anti-biotin antibodies. The band for unbound P*oprH* is visible at the bottom of each image and indicated by an arrow. Grey arrow indicates unbound His$_6$V5-tagged BrlR. Black arrow near the top of the image indicates shift. Experiments were carried out in triplicate and representative images are shown.

SUPPLEMENTARY REFERENCES

1. **Caiazza NC, O'Toole GA.** 2004. SadB is required for the transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas aeruginosa* PA14. J. Bacteriol. **186:**4476-4485.
2. **Heydorn A, Nielsen AT, Hentzer M, Sternberg C, Givskov M, Ersboll BK, Molin S.** 2000. Quantification of biofilm structures by the novel computer program COMSTAT. Microbiology **146:**2395-2407.
3. **Liao J, Sauer K.** 2012. The MerR-like transcriptional regulator BrlR contributes to *Pseudomonas aeruginosa* biofilm tolerance. J. Bacteriol. **194:**4823-4836.